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TAXOGENETICS OF THE *SACCHAROMYCES* SENSU STRICTO YEASTS FROM WESTERN AND SOUTH AFRICA

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ABSTRACT - Using genetic hybridization analysis, we reidentified *Saccharomyces* sensu stricto strains isolated from soil in South africa (J.P. van der Walt, 1970) as two biological sibling species *S. cerevisiae* Hansen and *S. paradoxus* Batschinskaia. The latter was found for the first time in Africa. Three *Saccharomyces* strains isolated from different wines in Western Africa (A. Guillermond, 1914) belong to *S. cerevisiae* and harbor each unique set of sucrose fermenting polymeric *SUC1*, *SUC2* and *SUC3* genes.

RÉSUMÉ - Des souches de *Saccharomyces* sensu stricto isolées du sol en Afrique du Sud (J.P. van der Walt, 1970) ont été reidentifiées par analyse génétique comme deux espèces biologiques, *S. cerevisiae* Hansen et *S. paradoxus* Batschinskaia. La dernière espèce a été trouvée pour la première fois en Afrique. Trois souches de *Saccharomyces* isolées de vins différents en Afrique Occidentale (A. Guilliermond, 1914) appartiennent à *S. cerevisiae* et ont chacune l'ensemble unique des gènes polymériques *SUC1*, *SUC2* et *SUC3* pour la fermentation du saccharose.

KEY WORDS: *Saccharomyces paradoxus*, *S. cerevisiae*, taxonomy, electrophoretic karyotyping, *SUC* genes.

INTRODUCTION

Although natural and cultural yeasts of the genus *Saccharomyces* Meyen ex Hansen from Europe and Asia have been intensively studied during a century, there is a short information about *Saccharomyces* isolated from other continents (do Carmo Sousa, 1969, Phaff & Starmer, 1987, van der Walt, 1970). At the beginning of the century a well-known French mycologist A. Guilliermond (1914) described *Saccharomyces chevalieri*, *S. lindneri* and *S. manginii* species isolated from different wines in Western Africa. In 50th J.P. van der Walt isolated several soil strains of *S. cerevisiae* Hansen, *S. coreanus* Saito and *S. uvarum* Beijerinck in South Africa. Besides, wine strains of *S. capensis* v.d. Walt & Tscheuschner, *S. coreanus* and *S. uvarum* are known

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from that region and *S cerevisiae* strains were isolated from Bantu beer (van der Walt, 1970) Since that time many nomenclatural changes have been made in the genus *Saccharomyces* (Barnett, 1992). Without additional studies most of the species mentioned above cannot be assigned to any of the currently accepted biological species of the *Saccharomyces* sensu stricto group *S cerevisiae, S paradoxus* Batschinskaia and *S bayanus* Sacc (Naumov, 1987; Naumov et al , 1992a, b; Vaughan Martini, 1989, Vaughan Martini & Kurtzman, 1985, Vaughan Martini & Martini, 1987) Only genetic hybridization analysis or DNA/DNA reassociation are suitable for delimiting the sibling species.

In the present study we reidentified by genetical methods some yeast strains isolated by A Guilliermond and J P. van der Walt in Western and South Africa The strains are maintained at the Centraalbureau voor Schimmelcultures in Delft (List of cultures, 1990) Some of their genetic peculiarities have also been studied Among African natural *Saccharomyces* yeasts we found unique population of biological species *S. cerevisiae* and yeasts of biological species *S. paradoxus*

MATERIAL AND METHODS

Strains

The list of *Saccharomyces* strains studied and their origin are presented in Table 1 The reference strains for biological sibling species were as follows *S cerevisiae* - CBS 5287, ATCC 48498, X2180 1A and *S paradoxus* - CBS 5829 The reference strains, methods for cultivation and hybridization of yeasts have been described elsewhere (Naumov, 1987, Naumov et al , 1986) Hybrids of homothallic yeasts were obtained by "spore to spore" mating method using a micromanipulator

CHEF gel electrophoresis

Chromosomal DNAs were prepared as described by Naumov et al (1991) Agarose slices were washed in 0.05 M EDTA, pH 8 0, prior to fractionation of chromosomes by contour clamed homogenous electric field (CHEF) gel electrophoresis A CHEF-DR™II apparatus (Bio-Rad, USA) was used to separate the chromosomal DNAs Agarose plugs containing chromosomal DNA were loaded into wells of a 1% agarose gel in 0 5 x TBE (89 mM Tris, 89 mM borate, 20 mM EDTA, pH 8.2) Electrophoresis was carried out at 200 V and 14°C for 15h with a switching time of 60 s and then for 8h with a switching time of 90 s After electrophoresis the gels were stained with ethidium bromide for visualizing of the chromosomes A standard set of *S cerevisiae* YNN 295 chromosomes was obtained commercially (Bio-Rad)

Southern blot analysis

After soaking the gels in 0 25 M HCl for 30 min, chromosomal DNA separated by CHEF was denaturated, neutralized and transferred to nitrocellulose filters which were then baked at 80°C for 2h The *SUC2* probe was a 0 9 kb BamHI-HindIII fragment isolated from pRB117 (Carlson & Botstein, 1983) The probe was prepared mainly according to Maniatis et al (1982) and labeled with digoxigenin-11-dUTP using the Nonradioactive DNA Labelling Kit (Boehringer Manheim, FRG) Hybridization was performed in 5 x SSC containing 0 1% N-laurol sarcosine, 0 02% SDS and 1% blocking reagent at 68°C overnight after which the filters were washed twice with 2 x SSC containing 0.1% SDS at room temperature for 5 min and with 0 1 x SSC containing 0 1% SDS at 68°C for 15 min Detection of hybridization was done with

the Nonradioactive Kit. The filters were incubated in colour solution in the dark overnight.

RESULTS AND DISCUSSION

Monosporic cloning

In genetic hybridization analysis only fertile monosporic parent strains should be used. First, fertile homozygotic cultures of *Saccharomyces* sensu stricto were obtained from single ascospores of collection strains listed in Table 1. All strains studied showed high ascospore viability (89-100%) and were homothallic. For each strain 5-10 tetrads were dissected.

Genetic identification

Monosporic cultures of African *Saccharomyces* sensu stricto strains were crossed with the reference strains of *S. cerevisiae* and *S. paradoxus*. The species determination was judged on the basis of the viability of hybrid ascospores and the recombination of control markers (Table 2). Strains CBS 403, CBS 405 and CBS 2888 produced fertile hybrids with *S. cerevisiae* reference strain while their hybrids with *S. paradoxus* were sterile (Table 2). Thus, these strains belong to *S. cerevisiae* species. On the contrary, strain CBS 2908 can be assigned to *S. paradoxus* species as it yielded fertile hybrid only with *S. paradoxus* CBS 5829. In all intraspecies hybrids normal meiotic segragation of control markers was observed. Strain CBS 400 was not included in the crosses. Its belonging to the biological species *S. cerevisiae* can be determined on the basis of hybridization analysis carried out by Ö. Winge and C. Robert (1952).

Two strains CBS 400 and CBS 403 were previously studied by DNA/DNA reassociation. They showed high DNA homology with *S. cerevisiae* type culture (96% and 87%, respectively) (Vaughan Martini & Kurtzman, 1985; Vaughan Martini & Martini, 1987). Our genetic studies revealed in South Africa for the first time wild *S. paradoxus* (CBS 2908) and *S. cerevisiae* (CBS 2888) yeasts. Wild species *S. paradoxus* was previously isolated from a number of sites in Europe, Far East Asia and North America (Naumov, 1987; Naumov et al., 1992a, 1993; Vaughan Martini, 1989). Wild strains of *S. cerevisiae* occur very seldom in nature and were found in Japan, Russian Siberia and Finland (Naumov & Naumova, 1991; Naumov et al., 1992a; Naumov & Nikonenko, 1988).

Identification of *SUC* genes

The fermentation of the sugars, viz. sucrose, maltose, α-methylglucoside, melibiose and starch is controlled in the yeast *S. cerevisiae* by the gene families (Barnett, 1981; Carlson et al., 1985; Naumov et al., 1991; Needleman, 1991; Pretorius & Marmur, 1988; Winge & Roberts, 1958). Polymeric sugar genes are suitable as convenient markers for strain identification but not for species delimination (Naumov, 1985). The polymeric *SUC* gene family is known to contain 6 genes: *SUC1* (chromosome VII), *SUC2* (chromosome IX), *SUC3* (chromosome II), *SUC4* (chromosome XIII), *SUC5* (chromosome IV) and *SUC7* (chromosome VIII) (Carlson et al., 1985; Mortimer et al., 1992). Each of the *SUC* genes encodes β-fructosidase (invertase) hydrolyzing sucrose (Ottolenghi, 1971). Ö. Winge and C. Roberts (1952) found that a strain of *S. chevalieri* (CBS 400) harbored three polymeric genes *SUC1*, *SUC2* and *SUC3*. According to our preliminary data strain CBS 405 had several *SUC* genes (Naumov, 1972). In this

Table 1 - Strains of *Saccharomyces* sensu stricto from which monosporic cultures were used

Tableau 1 - Liste des souches de *Saccharomyces* sensu stricto

Species (original) designation	Strain designation	Source	Author
S. chevalieri	CBS 400^T	palm wine, Ivory Coast	A. Guillermond
S. lindneri	CBS 403^T	ginger wine, West Africa	A. Guillermond
S. mangini	CBS 405^T	billi wine, West Africa	A. Guillermond
S. coreanus	CBS 2888	soil, South Africa	J.R. van der Walt
S. cerevisiae	CBS 2908	soil, South Africa	J.R. van der Walt
S. paradoxus	CBS 5829	soil, Denmark	V. Jensen
S. cerevisiae	ATCC 48498	wine, Carpathians Mountains, Ukraine	L.V. Turina
S. cerevisiae	CBS 5287	grape berries, Far East of Russia	I.A. Mazilkin
S. cerevisiae	YNN 295	genetic line	D. Vollrath & R.W. Davis
S. cerevisiae	X2180-1A	genetic line	R.K. Mortimer

Strain CBS 400 used was not monosporic. CBS 403 = VKM Y-407, CBS 405 = VKM Y-481, CBS 2888 = NRRL 12638, CBS 5287 = VKM Y-502, ATCC 48498 = M 437. ATCC = American Type Culture Collection, Rockville, U.S.A. CBS = Centraalbureau voor Schimmelcultures, Delft, Holland. M = Magarach Institute of Viticulture and Wine Making, Yalta, Ukraine. NRRL = Northern Regional Research Laboratories Peoria, Ill., U.S.A. VKM = All-Russian Collection of Microorganisms, Moscow, Russia. T = type culture.

connection, it was interesting to investigate the *SUC* genotypes of the *S. cerevisiae* strains isolated from Africa.

Chromosomal DNAs of the strains studied were separated by pulsed field gel electrophoresis (Fig. 1A). Strains CBS 400, 403 and 405 revealed karyotyping patterns similar to one another and to references strain X2180-1A (Fig. 1A, lanes 3-5 and lane 2, respectively). Strain X2180-1A represents a 'wild type' (in genetic terms) of *S. cerevisiae* karyotype (Naumov et al., 1992b). Following electrophoresis the chromosomal DNAs were transferred to nitrocellulose filter and hybridized with the *SUC2* probe. In

Table 2 - Genetic analysis of the hybrids of the biological species *S. cerevisiae* (CBS 403, CBS 405, CBS 2888, ATCC 48498, X2180-1A and CBS 5287) and *S. paradoxus* (CBS 2908 and CBS 5829).

Tableau 2 - Analyse génétique d'hybrides de *S. cerevisiae* (CBS 403, CBS 405, CBS 2888, ATCC 48498, X2180-1A et CBS 5287) et *S. paradoxus* (CBS 2908 et CBS 5829).

Origin of hybrids	No. of spore pairs crossed	No. of zygotes obtained	No. of tetrads isolated	Proportion of viable ascospores of hybrids (%)	Segregation of control markers (+:-)
		S. cerevisiae x S. cerevisiae			
403 x 48498	30	3	21	90	2:2 (15)
405 x 48498	35	5	22	83	2:2 (13)
2888 x X2180	55	3	29	42	24:21
		S. paradoxus x S. paradoxus			
2908 x 5829	69	6	29	66	39:38
		S. cerevisiae x S. paradoxus			
403 x 5829	39	10	25	0	-
405 x 5829	5	1	27	0	-
5287 x 2908	37	5	23	0	-
2888 x 5829	53	12	28	0	-

Segregation of the control markers is in accordance with data of random spore or tetrad analysis. Number of tetrads is indicated in paranthesis. Reference strain no. 5829 was marked by a UV-induced adenine (*ade*) auxotrophy (red colonies). Strains no. 403, 405, 48498, 2888 and X2180-1A have natural markers Mal$^+$, Mal$^+$, Mal$^+$, Gal$^+$ and Gal$^+$, respectively.

strains CBS 400, CBS 403 and CBS 405 isolated from different wines (Table 1), the *SUC2* probe hybridized to three different bands (Fig. 1B, lanes 3-5). Comparing with standard strain YNN 295 having known order and sizes of chromosomes, these bands correspond to chromosomes VII, II and IX to which the *SUC1*, *SUC2* and *SUC3* genes respectively map. Additionally, the gene probes *LYS2* (chromosome II) (Eibel & Philippsen, 1983), *LYS1* (chromosome IX) and *TRP5* (chromosome VII) (Balzi et al., 1987) showed hybridization to the same bands as *SUC2* probe did (data not shown). Taking the data mentioned above into account, strains CBS 403 and CBS 405 are more likely to have the same genotype as CBS 400 *SUC1 SUC2 SUC3*. Strain CBS 2888 isolated from soil had only one *SUC* gene on chromosome IX (Fig. 1B, lane 6). The cross with the reference strain X2180-1A (*SUC2*) confirmed that the only *SUC* gene of strain CBS 2888 was allelic to *SUC2*, as no segregation of ability to ferment sucrose was found.

Recently, we have studied by karyotyping and Southern analysis the *SUC* genes of several dozens of natural *S. cerevisiae* strains isolated from different geo-

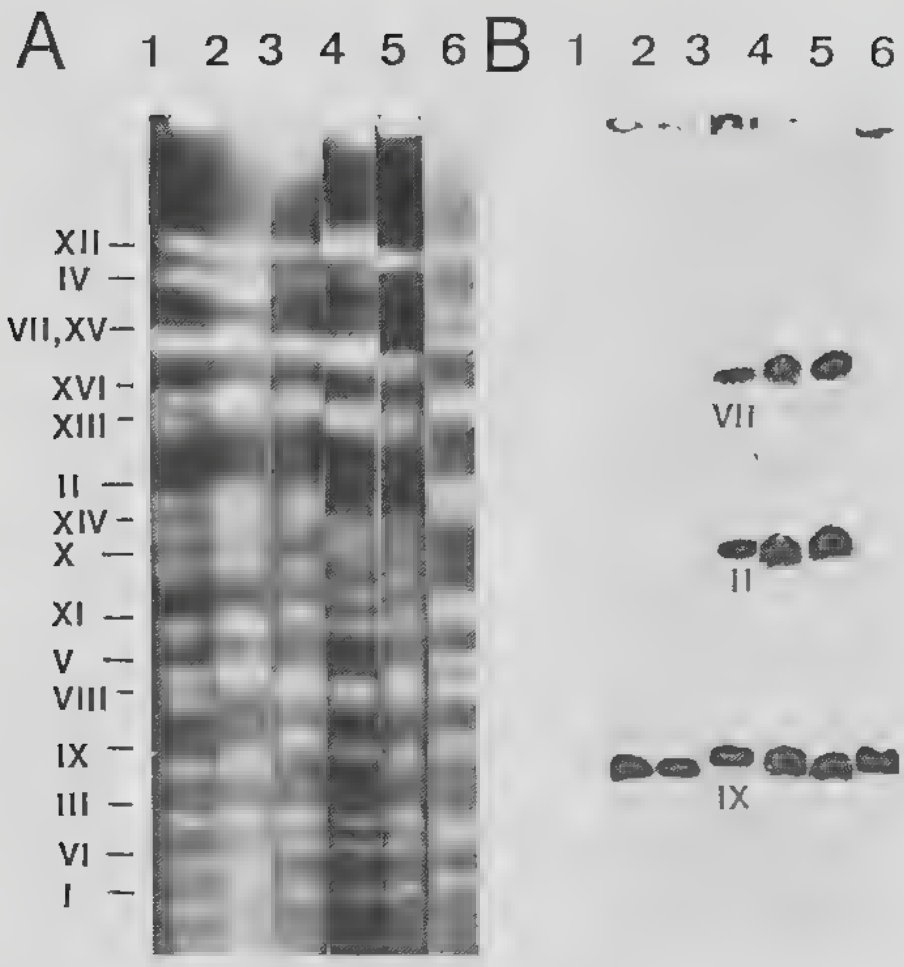

Figure 1 Southern hybridization analysis of chromosomal DNAs from African *S. cerevisiae* strains. Lane 1, YNN 295, lane 2, X2180-1A; lane 3, CBS 400, lane 4, CBS 403, lane 5, CBS 405, lane 6, CBS 2888. Ethidium bromide stained gel (A) corresponding to hybridization of chromosomal DNAs with the *SUC2* probe (B). The linkage group numbering refers to the chromosomes of the strain YNN 295.

Figure 1 Analyse Southern d'ADN chromosomique des souches de *S. cerevisiae* d'Afrique. Piste 1, YNN 295, piste 2, X2180-1A; piste 3, CBS 400, piste 4, CBS 403, piste 5, CBS 405, piste 6, CBS 2888. Gel teint au bromure d'éthydium (A) correspondant à l'hybridation d'ADN chromosomique avec la sonde *SUC2* (B).

graphic regions (data not shown). Most of sucrose fermenting strains showed one *SUC2* gene; strains non fermenting sucrose possessed the silent sequences *suc2*^{0}. It seems that at least at the beginning of this centuary in Western Africa there was an isolated population of yeast *S. cerevisiae* having an original set of *SUC* genes.

This study showed that populations of *Saccharomyces* occurring far from Europe can have unique genetic constitution. Probably, the enlarged geographic screening of natural *Saccharomyces* strains would allow revealing new genes in *S. cerevisiae*.

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